

What is claimed is:

1. A recombinant DNA molecule comprising at least a portion encoding subunit S1 of the Bordetella exotoxin, or a fragment or derivative of said portion, wherein said portion or fragment or derivative encodes a polypeptide having a biological activity which (a) can elicit toxin-neutralizing levels of antibodies and (b) is free of enzymatic activity associated with toxin reactogenicity.
2. The recombinant DNA molecule of claim 1 wherein said portion encoding said polypeptide further comprises a major epitope known to be important in providing immunoprotection against pertussis toxicity.
3. The recombinant DNA molecule of claim 1 wherein said toxin-neutralizing levels of antibodies provide immunoprotection against pertussis toxicity.
4. The recombinant DNA molecule of claim 1 wherein said biological activity of (b) is obtained by site-specific mutagenesis resulting in an analog of subunit S1 which is substantially inactive enzymatically.
5. The recombinant DNA molecule of Claim 4 wherein said S1 subunit comprises site-specific mutations of the S1 subunit in the region bounded by valine 7 and proline 14, inclusively.
6. The recombinant DNA molecule of claim 5 wherein said site-specific mutation occurs at the arginine 9 site.

7. The recombinant DNA molecule of claim 6 wherein arginine 9 is replaced with lysine.

8. The recombinant DNA molecule of claim 1 wherein said Bordetella exotoxin is selected from the group consisting of B. pertussis, B. parapertussis, and B. bronchiseptica.

Sub B2
9. An analog of Bordetella exotoxin S1 subunit, said analog having a biological activity which (a) can elicit toxin-neutralizing levels of antibodies and (b) enzymatic activities associated with toxin reactogenicity.

10. The analog of claim 9 wherein said analog further comprises at least one major epitope known to be important in providing immunoprotection against Bordetella toxicity.

2 11. The analog of claim 9 wherein said toxin-neutralizing levels of antibodies provide immunoprotection against Bordetella toxicity.

Sub B3
12. The analog of claim 9 wherein said biological activity of (b) is obtained by site-specific mutagenesis resulting in said analog being substantially inactive enzymatically.

13. The analog of claim 12, wherein said S1 subunit comprises site-specific mutations of the S1 subunit in the region bounded by valine 7 and proline 14, inclusively.

Sub B4
14. The analog of claim 13 wherein said site-specific mutagenesis occurs at the arginine 9 site.

15. The analog of claim 14, wherein arginine 9 is replaced with lysine.

16. The analog of claim 9 wherein said Bordetella exotoxin is selected from the group consisting of B. pertussis, B. parapertussis, and B. bronchiseptica.

17. The analog of claim 9 wherein said amino-terminus includes a methionylvalyl sequence.

18. An analog of Bordetella exotoxin subunit S1, said analog comprising an amino acid sequence as disclosed in Figure 7.

Sub B
19. An improved vaccine comprising a genetically-engineered subunit S1 of Bordetella exotoxin having a biological activity which (a) can elicit toxin-neutralizing levels of antibodies and (b) is free of enzymatic activity associated with toxin reactogenicity.

20. The improved vaccine of claim 19 wherein said subunit S1 includes at least one major epitope for providing immunoprotection against Bordetella toxicity.

21. The improved vaccine of claim 21 wherein said toxin-neutralizing levels of antibodies provide immunoprotection against Bordetella toxicity.

Sub B
22. The improved vaccine of claim 19 wherein said biological activity of (b) is obtained by site-specific mutagenesis resulting in an analog of subunit S1 which is substantially inactive enzymatically.

23. The improved vaccine of claim 22 wherein said site-specific mutagenesis is directed to the region bound by valine 7 and proline 14, inclusively,.

Sub B7
24. The improved vaccine of claim 23 wherein said site-specific mutagenesis is directed to the arginine 9 site.

Sub C4
25. The improved vaccine of claim 24 wherein arginine 9 is replaced with lysine.

26. The improved vaccine of claim 19 wherein said Bordetella exotoxin is selected from the group consisting of B. pertussis, B. parapertussis and B. bronchiseptica.

27. The improved vaccine of claim 19 further including at least one of said subunits S2, S3, S4, and S5, and mixtures thereof, of Bordetella exotoxin.

Sub B8
28. The improved vaccine of claim 25 wherein at least one of said subunits S2, S3, S4 and S5, and mixtures thereof, of Bordetella exotoxin is genetically engineered.

29. The improved vaccine of claim 19 wherein said genetically-engineered subunits S2, S3, S4 and S5 are expressed as non-fusion proteins in recombinant hosts selected from the groups consisting of E. coli., S. cerivisiae, Salmonella typhimurium, Salmonella typhi, Baccillus sp. and vaccinia.

Sub B9
30. The improved vaccine of claim 9 wherein said genetically-engineered subunits S2, S3, S4 and S5 include analogs of subunits S2, S3, S4 and S5 which have retained their ability to elicit toxin-neutralizing levels of antibodies.

31. A recombinant DNA molecule comprising at least a portion encoding subunit S2 of Bordetella

exotoxin or a fragment or derivative of said portion wherein the fragment or derivative encodes a peptide being a methionine-mature analog of subunit S2.

32. A recombinant DNA molecule comprising at least a portion encoding subunit S3 of Bordetella exotoxin or a fragment or derivative of said portion wherein the fragment or derivative encodes a peptide being a methionine-mature analog of subunit S3.

33. A recombinant DNA molecule comprising at least a portion encoding subunit S4 of Bordetella exotoxin or a fragment or derivative of said portion wherein the fragment or derivative encodes a peptide being a methionine-mature analog of subunit S4.

34. A recombinant DNA molecule comprising at least a portion encoding subunit S5 of Bordetella exotoxin or a fragment or derivative of said portion wherein the fragment or derivative encodes a peptide being a methionine-mature analog of subunit S5.

add
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